Br. J. Pharmac. (1978), **62**, 61–66

RELEASE OF MEDIATORS OF ANAPHYLAXIS: INHIBITION OF PROSTAGLANDIN SYNTHESIS AND THE MODIFICATION OF RELEASE OF SLOW REACTING SUBSTANCE OF ANAPHYLAXIS AND HISTAMINE

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1 When isolated perfused lungs from sensitized guinea-pigs were challenged with antigen, histamine, slow reacting substance of anaphylaxis (SRS-A) and prostaglandin-like substances were released into the effluent.

2 Treatment of the lungs before and during challenge with indomethacin (0.5–10 μg/ml), sodium aspirin (1–10 μg/ml), sodium meclofenamate (0.1–1 μg/ml) or ketoprofen (0.5–5 μg/ml) inhibited the release of prostaglandins while increasing the output of histamine and SRS-A between three- and five-fold.

3 Diethylcarbamazine (0.2–1 mg/ml) reduced the release of SRS-A and histamine but increased the amount of prostaglandin-like substances produced.

4 Eicosatetraynoic acid (10 μg/ml) inhibited formation of prostaglandins but did not modify release of histamine and SRS-A.

5 The results with non-steroid anti-inflammatory drugs and diethylcarbamazine suggest that prostaglandins, or some other product of the cyclo-oxygenase system, depress the anaphylactic release of SRS-A and histamine.

Introduction

Exogenous prostaglandins E_1, E_2 and $F_{2\alpha}$ depressed the release of histamine and slow reacting substance of anaphylaxis (SRS-A) from passively sensitized human lung during antigen challenge (Tauber, Kaliner, Stechschulte & Austen, 1973). When formation of endogenous prostaglandins was inhibited by treatment of passively sensitized human lung with the cyclo-oxygenase inhibitor, indomethacin, an increased amount of SRS-A was released during challenge (Walker, 1972). Similar potentiation of SRS-A release occurred in challenged bovine lungs treated with meclofenamate and aspirin (Burka & Eyre, 1975) and of histamine release from sensitized guinea-pig lungs treated with indomethacin (Gryglewski, Panczenko, Korbut, Grodzinska & Ocetkiewicz, 1975; Liebig, Bernauer & Peskar, 1975). These results suggest that products of metabolism of arachidonic acid by cyclo-oxygenase modulate the release of histamine and SRS-A during anaphylaxis in lung tissue.

We have investigated the effects of inhibition of the cyclo-oxygenase system on the release of histamine and SRS-A caused by anaphylactic shock in guinea-pig isolated perfused lungs and have shown that several non-steroid anti-inflammatory drugs increased the amount of SRS-A and histamine released during antigen challenge.

Part of this work has been communicated to the British Pharmacological Society (Engineer, Piper & Sirois, 1976).

Methods

Male guinea-pigs (Dunkin-Hartley strain) 200–250 g were sensitized with doses of egg albumin (Sigma, Grade II) 100 mg given subcutaneously and 100 mg intraperitoneally. Three weeks later the guinea-pigs

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were killed, the heart and lungs excised and rapidly transferred to the perfusion apparatus where they were immediately perfused with oxygenated Tyrode solution via the pulmonary artery. The lungs were initially perfused at 10 ml/min for not more than 5 min and then at 5 ml/minute. The lungs were inflated several times via the trachea until the effluent from the lungs was no longer blood-stained. The trachea was clamped with the lungs inflated. The lungs were suspended in a water-jacketed bath at 37°C and the top covered with cotton wool. The lungs were challenged with 10 mg egg albumin (Sigma Grade III) in 1 ml of Tyrode solution injected into the pulmonary artery. This was carried out in two stages: initially 0.5 ml, and then 10 s later, another 0.5 ml. The lung perfusate was collected for 10 min in siliconized or polythene flasks surrounded by ice. Two lungs were isolated and perfused simultaneously. One received an intra-arterial infusion of the drug under investigation, while the other was given an intra-arterial infusion of a solution of the vehicle used to prepare the drug solution and acted as the control lung. The drugs investigated were non-steroid anti-inflammatory drugs and eicosatetraynoic acid (ETA) to inhibit prostaglandin synthesis, diethylcarbamazine to inhibit SRS-A release (Orange, Valentine & Austen, 1968), arachidonic acid to release prostaglandins and related substances (Palmer, Piper & Vane, 1973) and prostaglandins E_2 and $F_{2\alpha}$. Most of the drug solutions were infused for 15 min before antigen challenge but arachidonic acid and ETA were infused for 30 minutes. Ovalbumin (10 mg) was then injected into the pulmonary artery and the effluent collected for 10 min into ice-cold flasks. The infusion of the drug solution was continued throughout the collection. The lung effluent was examined for its SRS-A, histamine and prostaglandin content as described below. The method described above is modified from that demonstrated to us by Dr W. E. Brocklehurst and colleagues (Brocklehurst, 1962).

Bioassay of SRS-A

SRS-A was assayed on stripped longitudinal smooth muscle of the guinea-pig ileum (Rang, 1964), superfused at 5 ml/min with oxygenated Tyrode solution warmed to 37°C. A solution of mepyramine maleate and hyoscine hydrobromide was infused into the Tyrode solution superfusing the tissues to give a final concentration of base 1×10^{-6} M. The SRS-A content of the lung effluent was estimated by immediately assaying aliquots of effluent against a partially purified laboratory standard preparation of SRS-A (Engineer *et al.*, 1976) and the results expressed in arbitrary units; the unit being defined in terms of the activity of an initial batch; 0.068 ± 0.004 unit SRS-A are equivalent to 10 ng histamine (equivalent to 2 units SRS-A expressed by the method of Stechschulte, Austen & Bloch, 1967). Relative threshold doses of SRS-A, prostaglandins E_2, $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$ required to contract guinea-pig ileum were measured. 4-Oxy-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712) (Augstein, Farmer, Lee, Sheard & Tattersall, 1973) 1 μg/ml was used to antagonize the action of SRS-A.

In some experiments base hydrolysis with NaOH (0.1 N) was used to destroy the smooth muscle contracting activity of E-type prostaglandins present in lung effluent which could interfere with the assay of SRS-A.

Radioimmunoassay of prostaglandins

The concentrations of prostaglandins E_2 or $F_{2\alpha}$ in lung effluent were measured by radioimmunoassay. Lung effluent was extracted twice with ethyl acetate at pH 3, evaporated to dryness under vacuum and kept at −20°C until required. The extract was redissolved in tricine-buffered saline (pH 8) for radioimmunoassay (Jose, Niederhauser, Piper, Robinson & Smith, 1976). Most of the assays were carried out using an antiserum to prostaglandin E_2. The percentage cross-reactions of this antiserum with other prostaglandins were: E_1, 44; $F_{2\alpha}$, 1.5; $F_{1\alpha}$, 0.6; A_2, 0.5; B_2, 0.04; D_2, 0.13; 15-keto-prostaglandin E_2, 19; 15-keto-13,14-dihydro-prostaglandin E_2, 0.2; 13,14 dihydro-prostaglandin E_2, 15; 6-keto-prostaglandin $F_{1\alpha}$, 1.3; thromboxane B_2, 0.2. The percentage cross-reactions of the antiserum to prostaglandin $F_{2\alpha}$ were: prostaglandins E_1 and E_2, 0.2 and 1.6 respectively; 15-keto prostaglandin $F_{2\alpha}$, 0.3; 15-keto-13,14, dihydro-prostaglandin $F_{2\alpha}$, 0.1; 13,14 dihydro-prostaglandin $F_{2\alpha}$, 4.8; 6-keto-prostaglandin $F_{1\alpha}$, 8.6. The percentage recovery during extraction was determined by the addition of 5000 d/min of [^{3}H]-prostaglandin E_2 (4 pg) to the lung effluent before extraction. The recovery of internal standard was 80–90%.

Fluorimetric analysis of histamine

Histamine content was assayed by an automated fluorimetric assay described by Evans, Lewis & Thompson (1973) and modified by Winsey (personal communication). Standard responses were obtained to 100–250 ng/ml of histamine. All substances infused through the lungs were tested for possible interference in the fluorimetric assay (with and without addition of histamine, 100 ng/ml). No interference was detected with any of the substances used.

Materials

The following drugs were used: aspirin, histamine acid phosphate, hyoscine hydrobromide (B.D.H.);

diethylcarbamazine citrate (Burroughs Wellcome); antiserum for prostaglandin E_2, ketoprofen, mepyramine maleate (May & Baker); indomethacin (Merck, Sharp & Dohme); sodium meclofenamate (Parke Davis); [^{3}H]-prostaglandin E_2 (160 Ci/mmol) and [^{3}H]-prostaglandin $F_{2\alpha}$ (160 Ci/mmol) (Radiochemical Centre, Amersham); arachidonic acid, egg albumin Grade II and III (Sigma); prostaglandins and metabolites (The Upjohn Company, Kalamazoo); 4-oxy-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712) (Fisons); eicosatetraynoic acid (ETA) (Roche Products); SRS-A and antiserum for prostaglandin $F_{2\alpha}$ (prepared in the department).

Results

Bioassay of SRS-A

The threshold dose of SRS-A causing contraction of stripped guinea-pig ileum was 0.012–0.062 units. This tissue was also contracted by prostaglandin E_2, 5–10 ng, prostaglandin $F_{2\alpha}$, 100 ng and 6-keto-prostaglandin $F_{1\alpha}$, 1 μg. The contractions to standard doses of SRS-A and to SRS-A present in the effluent were antagonized by the SRS-A antagonist, FPL 55712 1 μg/ml, whereas contractions to the above prostaglandins were not.

The effects of non-steroid anti-inflammatory drugs on the release of mediators during antigen challenge

Figure 1 shows the effects of indomethacin (0.5–10 μg/ml) on the release of SRS-A, histamine and prostaglandins during challenge of guinea-pig lungs. At all concentrations used, indomethacin caused a marked reduction of immunoreactive prostaglandins measured in terms of prostaglandin E_2. A similar decrease was shown when the prostaglandins were measured in terms of prostaglandin $F_{2\alpha}$ (control lungs, 89.7 ± 14.1; lungs treated with indomethacin, 1 μg/ml, 10.9 ± 0.8 ng equivalents $F_{2\alpha}$). Low doses (0.5–1 μg/ml) of indomethacin produced a marked increase in SRS-A and histamine release from guinea-pig lungs, while higher doses (5–10 μg/ml) caused a less than maximal increase in the output of mediators. Similar results were obtained with the other non-steroid anti-inflammatory drugs, sodium aspirin (1–10 μg/ml), sodium meclofenamate (0.1–1 μg/ml) and ketoprofen (0.5–5 μg/ml) as shown in Table 1.

Effect of arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$ on mediator release

During infusion of arachidonic acid (10 μg/ml) the level of prostaglandin-like activity assayed in terms of prostaglandin E_2 was increased from a control level of 79 to 333 ng equivalents per lung (result from pooled effluent of 5 lungs; because base hydrolysis was carried out on effluent taken through SRS-A purification). Infusion of arachidonic acid (5 μg/ml) increased the level of histamine released from a control level of 7.5 ± 1.7 to 21.6 ± 2.9 μg/lung, but the histamine release was unchanged from control values during infusion of arachidonic acid (10 μg/ml).

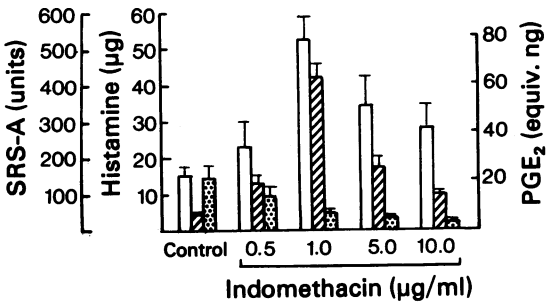

Figure 1 Effect of indomethacin on release of histamine, slow reacting substance of anaphylaxis (SRS-A) and prostaglandins. Indomethacin (0.5–10 μg/ml) was infused continuously before and during antigen challenge. Open columns: SRS-A (units); hatched columns: histamine (μg); stippled columns: prostaglandin expressed as ng equivalents of prostaglandin E_2. All quantities are the total amount present in the effluent from individual lungs collected for 10 min after antigen challenge. Each column represents the mean of 4–12 experiments. Vertical bars show s.e. means.

Infusion of prostaglandins E_2 and $F_{2\alpha}$ also caused an increase in histamine release. During infusion of 100 ng/ml of prostaglandin E_2, the release of histamine increased from a control of 7.5 ± 1.7 μg/lung to 17.1 ± 0.3 μg/lung. Also during infusion of 100 ng/ml of prostaglandin $F_{2\alpha}$, control values rose from 7.5 ± 1.7 to 25.3 ± 2.2 μg histamine/lung. In both cases, the SRS-A levels could not be reliably estimated owing to interference by the high level of prostaglandins in the lung effluent. Base hydrolysis destroyed the smooth muscle contracting activity of prostaglandin E_2 added to lung effluent, but prostaglandin $F_{2\alpha}$ is not affected by this process. The spasmogenic activity released by intra-arterial infusion of arachidonic acid was not removed by base hydrolysis.

Effect of eicosatetraynoic acid on mediator release

Infusion of ETA, 10 μg/ml into the lungs for 30 min before and during antigen challenge reduced the prostaglandin-like activity, assayed in terms of prostaglandin $F_{2\alpha}$, from 56.2 ± 9.0 to 6.0 ± 2.8 ng equivalents per lung but did not significantly alter the concentrations of histamine and SRS-A released.

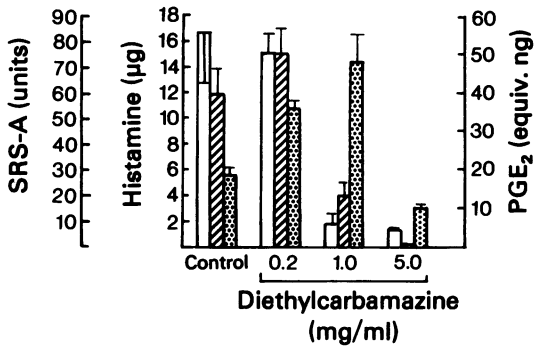

Figure 2 Effect of diethylcarbamazine on release of histamine, slow reacting substance of anaphylaxis (SRS-A) and prostaglandins. Diethylcarbamazine (0.2–5 mg/ml) was infused continuously before and during antigen challenge. Histogram is arranged and labelled as in Figure 1. Each column represents the mean of 4 experiments.

Effect of diethylcarbamazine on mediator release

The results in Figure 2 show that diethylcarbamazine (0.2–1 mg/ml) increased the amount of prostaglandin-like material released in anaphylaxis. Diethylcarbamazine (0.2 mg/ml) slightly reduced the concentration of SRS-A released and 1 mg/ml significantly inhibited the release of SRS-A. At this dose histamine output was also depressed. The release of all mediators was inhibited by 5 mg/ml diethylcarbamazine.

Discussion

The present results confirm and extend previous observations that non-steroid anti-inflammatory drugs increase the concentration of SRS-A and histamine released from sensitized guinea-pig lungs during antigen challenge while at the same time greatly decreasing the levels of prostaglandins released (Liebig *et al.*, 1975; Engineer *et al.*, 1976). They confirm those of Liebig *et al.* (1975) but are contrary to those of Mathé, Yen, Sohn & Hedqvist (1977) who used only doses of indomethacin approximately equivalent to the highest dose used in this paper. The actions of the aspirin-like drugs may also be due to interactions with cyclic nucleotides since, at high doses, non-steroid anti-inflammatory drugs inhibit other enzymes such as phosphodiesterase (Stefanovich, 1974). This would increase cyclic AMP levels, which may account for the less than maximal release of SRS-A and histamine observed with the highest doses used. At high concentrations of non-steroid anti-inflammatory drugs a similar decrease in histamine release was observed in rat peritoneal mast cells (Thomas & Whittle, 1976).

During anaphylaxis in guinea-pig perfused lung, a number of products of arachidonic acid metabolism are released; these include thromboxanes A_2 and B_2 prostaglandins E_2 and $F_{2\alpha}$, the 15-keto and 15-keto-13,14-dihydro-metabolites of prostaglandins E_2 and $F_{2\alpha}$, 6-keto prostaglandin $F_{1\alpha}$, 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT), 12L-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) (Hamberg, Svensson, Hedqvist, Strandberg & Samuelsson, 1976;

Table 1 Effect of aspirin, meclofenamate and ketoprofen on the release of mediators from guinea-pig lungs during challenge

Drug	*Concentration (μg/ml)*	*SRS-A (units)*	*Histamine (μg)*	*Prostaglandin-like activity (ng eq. PGE_2)*
Control	—	138 ± 26.0	4.4 ± 0.6	29.9 ± 4.6
Sodium aspirin	1.0	173.1 ± 25.1	13.9 ± 2.9	7.9 ± 1.2
	5.0	465 ± 62.1	19.5 ± 2.9	2.7 ± 0.4
	10.0	209 ± 30.6	12.2 ± 3.1	3.5 ± 0.3
Control	—	138 ± 26.0	4.7 ± 0.4	21.5 ± 2.9
Sodium meclofenamate	0.1	269 ± 75.0	6.9 ± 1.9	—
	0.5	333 ± 24.0	19.2 ± 4.5	4.1 ± 0.5
	1.0	159 ± 29.5	10.8 ± 2.6	—
Control	—	33.3 ± 12.3	2.4 ± 0.5	28.9 ± 7.2
Ketoprofen	0.5	368 ± 83.9	14.5 ± 3.7	4.4 ± 0.7
	1.0	165.3 ± 8.7	11.8 ± 4.2	2.3 ± 0.2
	5.0	50.8 ± 7.1	2,2 ± 0.1	1.5 ± 0.1

Number of experiments = 4–12. Values given per lung.

Dawson, Boot, Cockerill, Mallen & Osborne, 1976). Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ are metabolites of thromboxane A_2 and prostacyclin (PGI_2) (Moncada, Gryglewski, Bunting & Vane, 1976) respectively. Of the above substances there is evidence that prostaglandins E_2, $F_{2\alpha}$ and thromboxane B_2 may influence the release of SRS-A.

In the experiments described, the release of prostaglandin-like substances was measured as an indication of arachidonic acid metabolism. In most experiments the immunoreactive prostaglandin-like material released was measured in terms of prostaglandin E_2. The antiserum to prostaglandin E_2 had low cross-reactions with thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ which constitute 30% and 6% respectively of the prostaglandin-related material released (Dawson *et al.*, 1976). The appreciable cross-reactions with the metabolites of prostaglandin E_2 are acceptable because their presence is indicative of the initial release of the prostaglandin. Although the antiserum to prostaglandin $F_{2\alpha}$ showed appreciable cross-reaction with 6-keto-prostaglandin $F_{1\alpha}$, the results obtained with the aspirin-like drugs confirmed those obtained with the antiserum to prostaglandin E_2. Having established by means of ethyl acetate extraction and thin-layer chromatography that prostaglandins E_2 and $F_{2\alpha}$ were present in the effluent from the lungs used, the use of radioimmunoassay was a valid method of measuring the prostaglandin-like activity released from the lungs.

Although the guinea-pig ileum smooth muscle strip used to assay SRS-A also contracted to prostaglandins E_2, $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$, the relative threshold doses of these substances were much higher than that of SRS-A. Moreover, the use of FPL 55712 showed that these substances did not account for the contractions of this tissue caused by the lung effluent.

If the non-steroid anti-inflammatory drugs used in this study inhibit the action of cyclo-oxygenase (Lands, Le Tellier, Rome & Vanderhoek, 1974), they would be expected to prevent the formation of endoperoxides and therefore thromboxanes, prostaglandins and prostacyclin. However, it has been suggested that these drugs may act on other enzymes to inhibit preferentially the formation of prostaglandins and have less action on the formation of thromboxanes (Boot, Brockwell, Dawson & Sweatman, 1977).

The experiments with non-steroid anti-inflammatory drugs strongly suggest that although only the release of prostaglandins was measured, some product(s) of arachidonic acid metabolism formed via the cyclo-oxygenase pathway (see Hamberg, Svensson & Samuelsson, 1976) depresses the anaphylactic release of SRS-A and histamine. ETA which prevents metabolism of arachidonic acid by either cyclo-oxygenase or lipoxygenase, inhibited the release of prostaglandins but did not increase the output of histamine and SRS-A which confirms the findings of Boot *et al.* (1977). Tauber *et al.* (1973) showed that exogenous prostaglandins E_1, E_2 and $F_{2\alpha}$ depressed the release of histamine and SRS-A during antigen challenge of human chopped lungs and Boot *et al.* (1977) confirmed that prostaglandin E_2 inhibited the release of SRS-A in guinea-pig lung but found that prostaglandin $F_{2\alpha}$ stimulated its output. This group also showed that thromboxane B_2 in the amounts released from guinea-pig perfused lungs during anaphylaxis (up to 1 μg/ml) (Dawson *et al.*, 1976; Boot, Dawson, Cockerill, Mallen & Osborne, 1977) almost doubled the output of SRS-A from guinea-pig chopped lung. If thromboxane B_2 has the same action in perfused lungs and if non-steroid anti-inflammatory drugs do not readily inhibit the formation of thromboxanes, this compound may partly account for the increased release of SRS-A and histamine seen in the experiments described.

In contrast to endogenous products of arachidonic acid metabolism, neither exogenous arachidonic acid nor prostaglandins E_2 or $F_{2\alpha}$ depressed the anaphylactic release of histamine and SRS-A but increased the release of histamine. The infused arachidonic acid would be converted to thromboxanes which might stimulate the release of histamine. Also Tauber *et al.* (1973) have shown that low concentrations of prostaglandin E_1 and $F_{2\alpha}$ depressed cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels in human chopped lung and enhanced mediator release. Since the percentage conversion of arachidonic acid to prostaglandins in perfused lungs is very low (approximately 0.1%; calculated from Palmer *et al.*, 1973), the concentration of prostaglandins formed might have lowered the cyclic AMP levels.

Further evidence for the modulating role of prostaglandin-related materials in the lung was given by the fact that diethylcarbamazine decreased the levels of histamine and SRS-A released and at the same time increased the output of prostaglandins. When injected or infused into guinea-pig perfused lungs, histamine and SRS-A release rabbit aorta contracting substance (RCS) (a mixture of thromboxane A_2 and endoperoxides) and prostaglandin-like material (Palmer *et al.*, 1973; Liebig *et al.*, 1975; Engineer *et al.*, 1976; Engineer *et al.*, unpublished results) so that endoperoxides, thromboxanes, prostacyclin or prostaglandins released in anaphylaxis may influence further release of SRS-A and histamine, perhaps through an interaction with cyclic nucleotides. Further investigation is required to establish the relative importance of individual products of arachidonic acid metabolism.

The findings described in this paper help to explain the mechanism of action of non-steroid anti-inflammatory drugs in acute immunological reactions. The

fact that Miller & Robson (1976) observed a similar potentiation of anaphylactic bronchoconstriction by non-steroid anti-inflammatory drugs in the guinea-pig *in vivo* indicates that the modulating role of prostaglandin-related substances is important in the lungs both *in vivo* and *in vitro*.

We wish to thank Fisons Ltd. and the Conseil de la Recherche en Sante du Quebec for financial support, May & Baker Ltd. for the gift of antiserum to prostaglandin E_2 and ketoprofen, Merck, Sharp & Dohme for indomethacin, Parke Davis & Co. for sodium meclofenamate, Roche Products for ETA, Fisons Ltd for FPL 55712 and Dr J. E. Pike of the Upjohn Company, Kalamazoo for prostaglandins E_2 and $F_{2\alpha}$.

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(Received March 28, 1977.
Revised July 25, 1977.)